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Aged rats show dominant modulation of lower frequency hippocampal theta rhythm during running

Jia-Yi Li [a,b,c], Terry B.J. Kuo [a,b,d,e,f,g,h], Cheryl C.H. Yang [a,b,d,e,*]

[a] Institute of Brain Science, National Yang-Ming University, Taipei, Taiwan
[b] Sleep Research Center, National Yang-Ming University, Taipei, Taiwan
[c] Department of Health and Leisure Management, Yuanpei University of Medical Technology, Hsinchu, Taiwan
[d] Brain Research Center, National Yang-Ming University, Taipei, Taiwan
[e] Department of Education and Research, Taipei City Hospital, Taipei, Taiwan
[f] Institute of Translational and Interdisciplinary Medicine, National Central University, Taoyuan, Taiwan
[g] Department of Neurology, Taipei Veterans General Hospital, Taipei, Taiwan
[h] Division of Translational Medicine, Stroke & Neurovascular Center, Taipei Veterans General Hospital, Taipei, Taiwan

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ABSTRACT

Aging causes considerable decline in both physiological and mental functions, particularly cognitive function. The hippocampal theta rhythm (4–12 Hz) is related to both cognition and locomotion. Aging-related findings of the frequency and amplitude of hippocampal theta oscillations are inconsistent and occasionally contradictory. This inconsistency may be due to the effects of the sleep/wake state and different frequency subbands being overlooked. We assumed that aged rats have lower responses of the hippocampal theta rhythm during running, which is mainly due to the dominant modulation of theta frequency subbands related to cognition. By simultaneously recording electroencephalography, physical activity (PA), and the heart rate (HR), this experiment explored the theta oscillations before, during, and after treadmill running at a constant speed in 8-week-old (adult) and 60-week-old (middle-aged) rats. Compared with adult rats, the middle-aged rats exhibited lower theta activity in all frequency ranges before running. Running increased the theta frequency (Frq, 4–12 Hz), total activity of the whole theta band (total power, TP), activity of the middle theta frequency (MT, 6.5–9.5 Hz), and PA in both age groups. However, the middle-aged rats still showed fewer changes in these parameters during the whole running process. After the waking baseline values were substracted, middle-aged rats showed significantly fewer differences in ΔFrq, ΔTP, and ΔMT but significantly more differences in low-frequency theta activity (4.0–6.5 Hz) and HR than the adult rats did. Therefore, the decreasing activity and response of the whole theta band in the middle-aged rats resulted in dominant modulation of the middle to lower frequency (4.0–9.5 Hz) theta rhythm. The different alterations in the theta rhythm during treadmill running in the two groups may reflect that learning decline with age.

1. Introduction

Many brain functions, including responses to stimulation, sleep quality, memory, and cognitive function, are deficient in older animals (Blanco-Centurion and Shiromani, 2006; Tombaugh et al., 2002; van Praag et al., 2005). Dementia and cognitive impairment have continued to gain considerable attention (Ferri et al., 2005; Mukadam et al., 2015). Hippocampal theta band oscillations (4–12 Hz) are prominent during locomotion (Kahana et al., 2001; Vinogradova, 1995) and allude differences in the theta rhythm between passive and active waking (Bland, 1986; Orzel-Gryglewska et al., 2014; Vyazovskiy and Tobler, 2005). These oscillations might code information on motor behavior (Shin and Talnov, 2001). Studies have suggested that different types of locomotion lead to different hippocampal theta patterns (Arnolds et al., 1984; Gengler et al., 2005; Sinnamon, 2005). Furthermore, an increase in theta power is related to higher cognitive functioning and learning (Nokia et al., 2012; Olvera-Cortes et al., 2004), and lower theta activity can indicate aging-related cognitive impairment (Cummins and Finnigan, 2007; Olvera-Cortes et al., 2012). Adding the serotonin agonists in medial septum/diagonal band of Broca has also been shown to increase theta activity and enhance working memory (Lopez-Vazquez et al., 2014). However, running-related theta activity, which is related to the speed of locomotion (Li et al., 2012), does not seem to be affected by aging (Shen et al., 1997; Slawinska and Kasicki, 1998). Many extrinsic (*e.g.*, types and speed of locomotion) and intrinsic (*e.g.*, conscious states, aging, cognitive functioning) factors modulate or influence the

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[*] Corresponding author at: Institute of Brain Science, National Yang-Ming University, No. 155, Sec. 2, Li-Nong St., Beitou, Taipei 11221, Taiwan.
E-mail address: cchyang@ym.edu.tw (C.C.H. Yang).

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theta rhythm during behavior testing; therefore, the theta rhythm must be carefully controlled when studying the effects of aging.

In relation to the behavior of animals, two diverse types of hippocampal theta rhythms have been proposed: cholinergic-dependent activity (4–7 Hz, immobility-related theta, type 2 theta) and a noncholinergic component (6–9 Hz, voluntary motor theta or cognition-related theta, type 1 theta) (Kramis et al., 1975; Olvera-Cortes et al., 2004; Vanderwolf and Baker, 1986). During voluntary motor behavior, both type 1 and type 2 theta activities and frequencies are increased. Furthermore, studies have postulated the existence of an exploration-related sub-band of the theta frequency (Frq) ranging from 5 to 12 Hz (Gavrilov et al., 1996; Hinman et al., 2011; Vinogradova, 1995). Therefore, the whole theta band (4–12 Hz) can be divided into several different frequency sub-bands according to the different functions of the theta rhythm (Gutierrez-Guzman et al., 2011; Olvera-Cortes et al., 2002); however, the frequency range of different functions may be partially overlapped.

We have established a freely moving rat model that can be used to study various cerebral mechanisms simultaneously during treadmill exercise and wheel running, even in a pipe (Chen et al., 2011; Kuo et al., 2011; Li et al., 2012; Li et al., 2014). In this model, rats can run freely without physical restraint; in this context, some limits to their movement can result in discontinuity in their motion. According to previous studies (Kuo et al., 2010; Olvera-Cortes et al., 2012), investigating the hippocampal theta rhythm during running may provide crucial information on aging-related cognitive impairment. Furthermore, the sleep or wake state may produce larger fluctuations in the theta rhythm than experimental manipulations would. In this study, we measured the waking baseline values for comparison. We hypothesize that aged rats exhibit lower responses of the hippocampal theta rhythm during running than adult rats do. Different frequency subbands of the theta rhythm show different patterns between the two age groups during the whole running process, even during the initiation of running. The aging-related decrease in theta activity in aged rats is mainly due to the dominant modulation of cognitive-related Frq subbands. The aims of the present study are as follows: to explore whether these two age groups show different changes in the theta rhythm during initial running and subsequent running within a small window length; to determine whether adult and middle-aged rats show different changes in the theta rhythm, heart rate (HR), and physical activity (PA) during the whole running process; and to compare the changes in the theta rhythm of adult and middle-aged rats during running with those of the waking state.

2. Materials and methods

For the experiments, we used 8-week-old (young, $n = 15$) and 60-week-old (middle-aged, $n = 7$) male Wistar–Kyoto (WKY) rats. The rats were raised and adapted in a sound-attenuated room (22 ± 2 °C; 40%–70% humidity) under a 12-h light dark cycle (lights on from 11:00 to 23:00) with standard food (pellet) and water provided *ad libitum*. The detailed surgical procedures have been described previously (Li et al., 2014). One week before recording, the rats were implanted with electrodes to measure electrophysiological signals. On the day of electrode implantation, the adult and middle-aged rats were aged 7 and 59 weeks, respectively. Under pentobarbital anesthesia (50 mg/kg, i.p.), the depth of which was confirmed according to the paw pinch reflex, each rat was placed in a standard stereotaxic apparatus, and the dorsal surface of the skull was exposed and cleaned. Seven screws were fixed into the exposed skull surface to record the electroencephalogram (EEG). One of these screws served as the occipital electrode (2 mm lateral and 2 mm anterior to the lambda), and another screw served as the reference electrode (2 mm posterior to the lambda). A monopolar electrode composed of 230-μm Teflon-insulated stainless steel wire was stereotaxically and permanently implanted into the right hippocampal CA1 region (2.4 mm lateral and 4.3 mm posterior to the bregma and 2.4 mm from the dura) (Paxinos and Watson, 2006). The other four screws were used to fix the connector. Electromyogram (EMG) signals were recorded from two electrodes inserted into the dorsal neck muscles, and electrocardiogram (ECG) signals were recorded from two electrodes placed dorsally under the skin. All electrophysiological signals were transmitted to a connector fixed to the skull with dental acrylic. After surgery, the rats were treated with cephalexin hydrate (15 mg/kg, s.c., bid, Sigma-Aldrich) and carprofen (5 mg/kg, s.c., bid, Sigma-Aldrich), and individually housed in translucent cages for 1 week for recovery.

We used a treadmill model for exercise. After surgery and recovery, the rats were housed individually, and their 24-h baseline values were recorded in their individual cages. Before the running test, the rats were familiarized with the motorized treadmill (Model T510E, Diagnostic & Research Instruments CO., Taoyuan, Taiwan) by running on the treadmill for 10 min on 3 consecutive days (belt speed $= 8$ m/min). On the recording day, the rats were held manually while the wireless sensor was fixed to their heads, and they were placed on the treadmill during the last dark period (09:00–11:00 AM) under a dim red light (<1 lx). All electrophysiological signals were recorded for 120 min. According to the running time, we used 80 min of data for further analysis, from the moment the rats were placed onto the treadmill (Fig. 1). The rats may sleep while the treadmill belt was still; therefore, we scored the sleep status. During the first 20 min, the rats were allowed to become familiar with their surroundings in the treadmill (0 m/min). Subsequently, 30 min of moderate exercise (13 m/min) was performed by each rat, and they were then allowed 30 min of recovery (0 m/min). The treadmill belt could reach its default speed within 1 s. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the National Yang-Ming University (IACUC permit no. 981238).

Data acquisition and storage were similar to those previously described (Kuo et al., 2011). A wireless sensor ($25 \times 21 \times 18.5$ mm, 8.3 g, KY4C, K&Y Lab, Taiwan) was mounted on the head of the rat to acquire the ECG, EEG, and EMG signals, and three-dimensional acceleration. All electrophysiological signals were amplified 1000-fold. Three axis accelerations were detected using a triaxial accelerometer (ADXL330, Analog Devices Inc., Norwood, MA, USA) in the anteroposterior (X), mediolateral (Y), and vertical (Z) dimensions, each detecting acceleration from -3 to $+3$ gravity (G). The EEG signals and accelerations were differentially filtered (EEG: 0.16–48.00 Hz, acceleration: DC to 29 Hz) and synchronously digitized by an analog–digital converter with sampling rates of 125 Hz and 62.5 Hz, respectively. All digitized signals were then wirelessly transmitted to a nearby data recorder (KY3, K&Y Lab, Taiwan) for storage on a flash memory disk for off-line analysis.

We designed a customized computer program in Pascal language (Borland Pascal 7.0, Borland, USA) for analyzing bioelectric signals (Li et al., 2012). The digital signal processing of the bioelectric signals was similar to the procedures used in our previous studies (Kuo et al., 2004a; Kuo et al., 2004b). We used a 2-s window length to obtain a frequency resolution of 0.5 Hz, which was necessary to study the three sub-bands of the theta power. The EEG was resampled at 64 Hz and truncated into successive 2-s time segments (windows or epochs). A Hamming window was applied to each time segment to attenuate the leakage effect (Kuo and Chan, 1993). Then, our algorithm estimated the power density of the spectral components through a fast Fourier transform (FFT) function. The resulting power spectrum was corrected for attenuation from the sampling process and application of the Hamming window. For each time segment, we calculated the amplitude

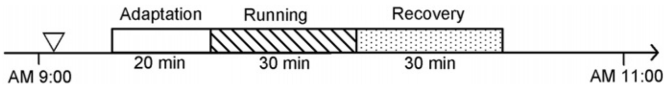

Fig. 1. Experimental schedule. Rats were held manually while the wireless sensors were fixed to their heads; subsequently, they were placed on the treadmill (inverted triangle, ▽).